Application of DNA barcoding identification kit to identify bark beetles of the genus *Dendroctonus* Erichson (Coleoptera: Curculionidae: Scolytinae)

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Abstract: 【Aim】 DNA barcoding has become a powerful tool in taxonomic identification. However, potential issues associated with DNA barcoding, such as overlap of genetic distances between intraspecific and interspecific variation, would directly affect the accuracy of identification. To accurately identify economically important *Dendroctonus* spp. intercepted at ports of entry, we employed a suite of nucleotide diagnostic sites as the identification characteristics (ICs). Each species is represented by a unique IC. 【Methods】 We designed genus-specific primers to improve amplification efficiency in *Dendroctonus*. Web-based software Gene Barcoding Analysis Software (GBAS) was designed to extract the IC from molecular sequences and a database was built specifically for species identification. Six unidentified specimens of *Dendroctonus* were also sampled to validate our IC approach. 【Results】 We obtained 325 bp of COI gene fragment (position 889 – 1 213 bp on the COI gene) using genus-specific primers. A combination of nucleotide diagnostic sites from the mitochondrial COI region of 12 species of *Dendroctonus* provides the IC, which is unique for each species, and thus can be used to differentiate closely related species. The ICs of six unidentified samples of *Dendroctonus* were compared to those of all invasive insects stored in the Plant Quarantine Organisms Identification System. The nucleotide sequence identity between the query sequence of *Dendroctonus* and IC database is 100% in all cases. 【Conclusion】 The results demonstrated that the IC approach can accurately identify the species of *Dendroctonus*. Our approach may be extended to barcode other economically important organisms and identify unknown samples.

Key words: Bark beetle; invasive species; DNA barcoding; COI; identification characteristics; GBAS

1 INTRODUCTION

DNA barcoding is a recently developed technique used to diagnose unidentified specimens or tissue samples (Hebert *et al.*, 2003a, 2003b; Hebert and Gregory, 2005). Three groups of analytical methods exist for DNA barcoding: phylogenetic tree-based approach, distance-based approach, and character-based approach (Sarkar *et al.*, 2002; Hebert *et al.*, 2003a; Hebert *et al.*, 2004; Rach *et al.*, 2008). The first two are the most popular but neither is a panacea for species identification (Hickerson *et al.*, 2006; Trewick, 2008; Yassin *et al.*, 2010). Distance-based approach suffers from the lack of a universal degree of DNA divergence (Meier, 2008); and tree-based approach encounters problems when a biological species cannot be represented by a monophyletic clade (*i. e.*, species are paraphyletic or polyphyletic) (Yassin *et al.*, 2010). Alternatively, a character-based approach was proposed, which can identify a suite of discrete molecular characters that can be used to unambiguously diagnose species (DeSalle *et al.*, 2005; DeSalle, 2007; Kelly *et al.*, 2007; Wong *et al.*, 2009). A combination of those substitutions can serve as the diagnostic characteristics for that species. For example, Wong *et al.* (2009) demonstrated the application of character-based barcoding approach in global shark fisheries. Character-based approach may even provide a higher resolution of identification than the other two methods when working with closely related species (Bergmann *et al.*, 2013). In order to accurately identify and intercept insects at ports of entry, we developed an insect DNA barcoding identification kit technology (IDK) (Ye *et al.*, 2015). This technology allows the quick and

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accurate identification of plant quarantine insects. A combination of nucleotide diagnostic sites from molecular sequences of closely related species of the same genus was generated and a database was built specifically for species identification.

Among economically important pests, bark beetles (Coleoptera, Curculionidae, Scolytinae) infest coniferous trees and represent a constant threat to forests (Wood *et al.*, 1982; Romon *et al.*, 2007). Particularly, members of the genus *Dendroctonus* Erichson are most destructive. This genus consists of 19 species, 17 of which are distributed in North America and 2 are distributed in Europe and Asia (Yin, 1984; Díaz *et al.*, 1998). All species in this genus infest conifers, primarily on *Pinus*, *Picea*, *Pseudotsuga*, and *Larix*. The adult beetles bore through the bark of the host where they feed on the phloem tissue (Wood, 1963).

Inadvertent introduction of invasive pests through international trade is a serious threat to native ecosystems and national economies (Cognato *et al.*, 2005). The red turpentine beetle (*D. valens* LeConte), native to North and Central America, was introduced to China and has been considered a dangerous pest since 1998 (Cognato *et al.*, 2005; Cai *et al.*, 2008). This species devastated coniferous forests in Shanxi Province and has spread into the adjacent forests in Shaanxi, Hebei, and Henan Provinces since 1999. More than 5 000 hm^2 of pine forests have been attacked and over 10 million trees of *Pinus tabulaeformis* have been killed (Li *et al.*, 2001; Miao *et al.*, 2001; Yan *et al.*, 2005).

Species of *Dendroctonus* are difficult to be identified due to their relatively small body size and great resemblance in external morphology. This challenge can be circumvented by the application of DNA barcoding (Wong *et al.*, 2009). Distance-based approach (*e. g.*, the Barcode of Life Data Systems) was not used due to its inherent ambiguity associated with distance threshold and lack of high level of specificity needed for regulatory purpose (Wong *et al.*, 2009). Tree-based approach is also insufficient for *Dendroctonus* because some species are paraphyletic with respect to their sister species (unpublished data). Instead, we looked for a combination of nucleotide diagnostic sites as the identification characteristics (ICs) of species of *Dendroctonus*, which should be conserved within species but variable among species. The IC is generated by the Gene Barcoding Analysis Software (GBAS) in Plant Quarantine Organisms Identification System (State Key Quarantine Laboratory of Forest and Germplasm Resources). The results showed that IC could differentiate the closely related species of *Dendroctonus* and accurately identified unknown samples from this genus.

2 MATERIALS AND METHODS

2.1 Sample preparation

Seven species of *Dendroctonus* frequently intercepted at various ports in Jiangsu Province were selected in this study (Table 1). All specimens were identified to species by YANG Xiao-Jun and AN Yu-Lin. Additional 273 sequences from 12 species of *Dendroctonus* were downloaded from GenBank (Tables 2 and 3). Six unidentified specimens of *Dendroctonus* were also sampled to validate our IC approach. Genomic DNA was extracted from a single leg removed from specimens using the magnetic beads method (Animal Tissue/Cell Genomic DNA Isolation Kit, Beijing GenMagBio Co., Ltd., China, www. genmagbio. com. cn/site) and stored at −20℃ (Chang *et al.*, 2013). Genus-specific primers were designed to amplify a partial sequence of the mitochondrial COI region of *Dendroctonus*. The forward primer (ZINK2) was [F] 5′-TAGATGTGG ACACCCGAGCCT-3′; and the reverse primer (ZINK3) was [R]5′-AGTTAGTCCTGCGAAGAG-3′.

2.2 DNA amplification and sequencing

Each PCR reaction was 25 μL, which consisted of 16.3 μL of ddH_2O, 2.5 μL of 10 × Taq buffer, 2 μL of 25 mmol/L $MgCl_2$, 2 μL of 10 mmol/L dNTP mixture, 0.5 μL of each primer (20 mmol/L), 0.2 μL of Taq polymerase (5 U/ μL, TaKaRa, www. takara-bio. com) and 1 μL of DNA template. Amplification was conducted under the condition of initial denaturation at 94℃ for 5 min, followed by 35 cycles of denaturation at 94℃ for 30 s, annealing at 47℃ for 45 s, and extension at 72℃ for 45 s, and a final extension at 72℃ for 10 min (Chang *et al.*, 2013). PCR products were separated by electrophoresis on a 1.5% agarose gel containing ethidium bromide. Purified PCR products were sequenced for both directions at the Genscript (Nanjing) Co., Ltd. (www. genscript. com) using the same PCR primers. All sequences in this study have been deposited in GenBank (Table 1).

2.3 Sequence analyses

Sequences were aligned using the Clustal W function in MEGA 5.0 (Kumar *et al.*, 2008) with default parameters. The COI alignment was trimmed to 325 bp. We used MEGA to check whether the sequences can be correctly translated into protein to ensure the authenticity of the *COI* gene. The complete sequence of *COI* gene (full length 1 545 bp, GenBank accession no. JQ005145) of *D. valens*

Table 1 Collection information of samples of *Dendroctonus* spp. used in this study

No.	Specimens	Ship origin	Intercepting port	Collecting date	GenBank accession no.
1	*D. adjunctus* 1	America	Changshu, Jiangsu	2005.07	KF207771
2	*D. adjunctus* 2	America	Lianyungang, Jiangsu	2005.03	KF766060
3	*D. micans*	Russian	Yangzhou, Jiangsu	2012.01	KF766062
4	*D. ponderosae*	America	Changshu, Jiangsu	2012.12	KF207772
5	*D. pseudotsugae* 1	Canada	Lianyungang, Jiangsu	2010.04	KF766055
6	*D. pseudotsugae* 2	America	Taicang, Jiangsu	2012.09	KF766056
7	*D. pseudotsugae* 3	America	Lianyungang, Jiangsu	2010.06	KF766057
8	*D. punctatus* 1	America	Yancheng, Jiangsu	2013.07	KF766058
9	*D. punctatus* 2	Canada	Taicang, Jiangsu	2013.08	KF766059
10	*D. rufipennis* 1	America	Changshu, Jiangsu	2010.10	KF766063
11	*D. rufipennis* 2	Canada	Taicang, Jiangsu	2011.01	KF766064
12	*D. simplex*	Canada	Jiangyin, Jiangsu	2011.10	KF766061
13	*D. valens* 1	n. a.	Jinzhong, Shanxi (not a port)	2012.05	KF766065
14	*D. valens* 2	n. a.	Jinzhong, Shanxi (not a port)	2004.07	KF766066
15	*D. valens* 3	n. a.	Jinzhong, Shanxi (not a port)	2011.08	KF766067
16	*D.* sp. #1	America	Taicang, Jiangsu	2014.02	KP893622
17	*D.* sp. #2	America	Taicang, Jiangsu	2013.05	KP893623
18	*D.* sp. #3	America	Yangzhou, Jiangsu	2014.02	KP893624
19	*D.* sp. #4	Canada	Taicang, Jiangsu	2011.05	KP893625
20	*D.* sp. #5	Canada	Changshu, Jiangsu	2011.01	KP893626
21	*D.* sp. #6	Canada	Taicang, Jiangsu	2011.01	KP893627

D. sp.: Unidentified samples used to validate the IC approach. n. a.: Not applicable.

Table 2 Number of COI sequences used for primer design and sequence analysis

Species	COI sequences obtained in this study	COI sequences from GenBank	Total
D. adjunctus	2	1	3
D. brevicomis	0	1	1
D. mexicanus	0	27	27
D. micans	1	4	5
D. ponderosae	1	56	57
D. pseudotsugae	3	84	87
D. punctatus	2	0	2
D. rhizophagus	0	22	22
D. rufipennis	2	40	42
D. simplex	1	1	2
D. terebrans	0	3	3
D. valens	3	34	37
Total	15	273	288

was used to locate positions of nucleotide sites.

For character-based barcode analysis, the combination of nucleotide diagnostic sites that are conservative within species but variable among species is recorded as the IC for each species. Software was developed called Gene Barcoding Analysis Software (GBAS, http://www.exoticorganism.com/comparegeneen.aspx), which imports FASTA-formatted DNA sequences and exports IC. Intraspecific variation was excluded. The software is web-based and written in C++. If provided with a query sequence from unknown specimen, the software attempts to identify query sequence to species in the database based on the IC.

We briefly introduce the algorithm of GBAS here using a simplified example. Suppose we have extracted IC for each species in genus X after comparing 35 bp of COI sequences of all species in that genus. The IC of species Y is as following:

3: G, 6: C, 9: C, 14: A, 28: G, 33: A.

It means that at site 3 for this given 35 bp region of *COI*, all individuals of species Y have a nucleotide G, and so on. When a query sequence from an unidentified sample is compared against the IC database, first it is necessary to know whether the query sequence represents the exact same COI region where IC was extracted. Namely, the 3rd nucleotide of the query sequence has to be homologous to (3: G) of species Y. If yes, subsequent IC can be compared to the corresponding sites of the query sequence.

However, query sequences rarely represent the same region of *COI* that we used to generate the IC. Even amplified by the same PCR primers, the query sequence can be longer or shorter. See examples below:

COI region used to extract IC (35 bp): 5′-GGGCACAGCCCTAAGCCTTCTAATCCGGGCCGAAC-3′.

Query sequence #1: 5′-GCCCTAAGCCTTCTAATCCGG-3′.

Query sequence #2: 5′-GCATAGTGGGCACAGCCCTA AGCCTTC-3′.

Table 3　Accession numbers for 273 COI sequences of *Dendroctonus* spp. obtained from GenBank

Species	COI sequences from GenBank	GenBank accession no.
D. adjunctus	1	AF067992
D. brevicomis	1	AF067999
D. mexicanus	27	AF067988, DQ022214 – DQ022218, DQ022220 – DQ022222, DQ022224, DQ022225, DQ022227 – DQ022229, DQ022231, DQ022233, DQ022237, DQ022240, DQ022241, DQ022243, DQ022247, DQ022250, DQ022251, DQ022254, DQ022260, DQ022264, DQ022265
D. micans	4	AF296554 – AF296557
D. ponderosae	56	DQ865977, DQ865978, DQ865980, DQ865981, DQ865983 – DQ865994, DQ865997 – DQ866003, DQ866005, DQ866012, DQ866013, DQ866016, DQ866017, DQ866021, JQ308438, JQ308442, JQ308445, JQ308447 – JQ308449, JQ308451, JQ308454, JQ308455, JQ308458 – JQ308460, JQ308460, JQ308462, JQ308465, JQ308467, JQ308474 – JQ308477, JQ3084779, JQ308481, JQ308486, JQ308488, JQ308493 – JQ308495, JX424213
D. pseudotsugae	84	EU043406 – EU043408, EU043410, EU043412 – EU043414, EU043421 – EU043429, EU043433 – EU043435, EU043437, EU043439, EU043440, EU043442 – EU043445, EU043447, EU043448, EU043451, EU043460, EU043462, EU043463, EU193124 – EU193129, EU193131, EU193132, EU193134, EU193138 – EU193147, EU193150 – EU193152, FJ174746, FJ174748, FJ174749, FJ174751, FJ174752, FJ174755, FJ174757 – FJ174759, FJ174762, FJ174763, FJ174765, FJ174766, FJ174768, FJ174770, FJ174774 – FJ174777, FJ174780 – FJ174783, FJ174787 – FJ174791, AF067995, JX424214
D. rhizophagus	22	AF067993, JQ005114, JQ005116, JQ005119, JQ005120 – JQ005126, JQ005129, JQ005134 – JQ005136, JQ005138 – JQ005144
D. rufipennis	40	DQ165383 – DQ165386, DQ165388 – DQ165402, DQ165404 – DQ165406, DQ165408, DQ165410 – DQ165412, DQ165416, DQ165418, DQ165420 – DQ165425, DQ165430, DQ165442, DQ165445 – DQ165449
D. simplex	1	AF067985
D. terebrans	3	JQ005146, AF068003, AF375315
D. valens	34	EU404096 – EU404100, JQ005145, AY724546, AY724556, AY724568, AY724569, AY724573, AY724574, AY724581, AY7245482, AY724584, AY724587, AY724589, AY724592, AY724611, AY724615, AY724621, AY724638, AY724645, AY724648, AY724650, AY724653, AY724655, AY724662, AY724667, AY724668, AY724671

Query sequence #3: 5′-CTTCTAATCC GGGCCG AACTAAGTCAA-3′.

Therefore, given various relative positions between the region used to extract IC and the query sequence (*e. g.*, #1, #2, #3) on the COI gene, we used a sliding window method to compare the query sequence and IC. The query sequence slides from 3′ end to 5′ end of IC. To identify query sequence #1:

COI region used to extract IC (35 bp): 5′-GG GCACAGCCCTAAGCCTTCTAATCCGGGCCGAAC-3′.

Query sequence #1: 5′-GCCCTAAGCCTTCTAAT CCGG-3′.

Step 1: transform the region used to extract IC to show only IC, other nucleotides are replaced by N: 5′-NN GNNCNNCNNNNANNNNNNNNNNNNNGNNNANNN-3′.

Step 2: pick the first nucleotide of query sequence #1 (G) and align it to the last nucleotide (N) of the above transformed IC sequence:

5′-NNGNNCNNCNNNNANNNNNNNNNNNNNGNNNANNN-3′.

5′-G-3′.

Step 3: create a dummy sequence by adding N to the 5′ end of G until the same length of transformed IC sequence is reached:

5′-NNGNNCNNCNNNNANNNNNNNNNNNNNGNNNANNN-3′.

5′-NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNG-3′.

Step 4: compare nucleotide site 3, 6, 9, 14, 28, and 33 between the two sequences and calculate match percentage. In this case, match percentage is zero (N-N is not considered a match):

Match percentage = matching number of ICs/ total number of ICs.

Step 5: repeat the process by adding one more nucleotides of query sequence #1 from its 5′ end (*e. g.*, G and C) and align them to the last two nucleotides of the transformed IC sequence. Calculate match percentage again. After all nucleotides of the query sequence have slidden in, N needs to be added at both ends to reach the same length as the transformed IC sequence.

Steps 2 – 5 were repeated until all nucleotides of the query sequence have slidden through the 5′ end of the transformed IC sequence. The selected alignment of query and IC sequences is the one that matches 100%. If no perfect match is found, the query specimen is considered inconclusive. To test the accuracy of our approach, we amplified and sequenced six unidentified samples of *Dendroctonus* with the same primer pair and query those sequences against the database.

3 RESULTS

3.1 DNA sequences

The genus-specific primer pair (ZINK2 and ZINK3) designed in this study successfully amplified all samples of *Dendroctonus*. We obtained 325 bp of COI gene fragment (position 889 – 1 213 on the COI gene). No insertions, deletions, or pre-mature stop codons were found in the protein-coding region.

3.2 Identification characteristics and species identification

Identification characteristics were generated for 12 species of *Dendroctonus* based on 288 sequences (Fig. 1). The GBAS detected a total of 59 nucleotide sites that are conserved within species but variable among species of *Dendroctonus*. Each of the 12 species could be represented by a unique combination of IC. Therefore, closely related species can be identified.

The ICs from six unidentified samples of *Dendroctonus* were compared to those from all invasive insects stored in the Plant Quarantine Organisms Identification System. The nucleotide sequence identity between the query sequence from *Dendroctonus* and IC database is 100% in all cases (Fig. 2). Species-level comparison indicated that two query sequences fully matched with the IC from *D. valens*, one sequence fully matched with the IC from *D. pseudotsugae*, and three sequences fully matched with the IC from *D. rufipennis*. These identifications were confirmed by later morphological examination. This result demonstrates that the IC approach can diagnose species of *Dendroctonus*.

id	insect	P4	P18	P21	P28	P30	P33	P36	P42	P45	P57	P60	P63	P69	P82	P102	P105	P108	P110	P112	P117	P120	P121	P123	P126	P127	P129	P132	P135	P141	P160	P168	P169
1	Dendroctonus_punctatus	G	A	C	A	A	C	C	A	T	A	C	A	A	C	A	A	T	C	T	C	T	T	T	T	C	T	A	T	A	A	A	T
2	Dendroctonus_rufipennis	G	A	C	A	A	C	C	A	T	A	C	A	A	C	A	A	T	C	T	C	T	T	T	T	C	T	A	T	A	A	A	T
3	Dendroctonus_pseudotsugae	G	A	C	A	A	C	T	A	T	T	C	A	A	T	A	A	T	C	C	T	T	T	T	T	C	T	G	T	A	A	T	C
4	Dendroctonus_terebrans	G	A	C	A	A	T	T	G	T	A	T	A	A	C	C	A	C	G	T	C	A	T	T	C	C	T	A	T	A	A	A	T
5	Dendroctonus_micans	G	A	T	A	A	T	C	A	T	T	T	A	A	C	A	A	T	C	C	T	C	C	T	T	C	T	A	T	C	A	T	C
6	Dendroctonus_ponderosae	G	A	C	A	A	T	T	A	T	A	C	A	A	T	T	A	T	C	T	T	C	T	C	C	C	T	A	T	A	T	A	T
7	Dendroctonus_rhizophagus	G	A	C	A	A	T	T	A	T	T	C	A	A	T	C	A	C	T	T	C	T	T	C	T	C	T	A	T	A	A	C	T
8	Dendroctonus_brevicomis	G	A	T	A	A	C	C	A	T	T	A	A	G	C	C	A	C	C	T	C	T	T	A	T	C	A	G	T	A	A	A	T
9	Dendroctonus_adjunctus	G	T	T	A	G	A	T	A	T	C	T	A	A	C	C	A	T	C	C	C	C	T	A	T	T	A	G	T	A	A	A	T
10	Dendroctonus_simplex	A	G	C	C	G	T	T	A	T	T	A	A	A	T	A	G	T	C	T	C	C	T	C	T	C	T	C	C	A	C	A	A	G	T
11	Dendroctonus_valens	G	A	C	A	A	T	T	A	T	T	G	A	A	C	C	A	C	G	C	T	T	T	C	T	C	A	A	T	A	A	T	T
12	Dendroctonus_mexicanus	G	C	C	A	A	A	T	A	C	T	T	T	A	C	C	A	T	C	T	C	C	T	G	T	T	A	G	T	T	A	T	T

P171	P174	P180	P184	P189	P192	P195	P198	P204	P207	P231	P237	P243	P255	P258	P270	P273	P274	P280	P289	P291	P292	P309	P312	P313	P319	P324
G	C	A	C	T	C	T	A	T	T	A	C	C	A	T	A	T	G	C	A	C	A	T	C	G	C	T
G	C	A	C	T	C	T	A	T	T	A	C	C	A	T	A	T	G	C	A	C	A	T	T	A	C	T
A	A	G	T	T	T	A	A	T	T	A	T	T	A	T	A	T	G	T	A	T	A	T	T	A	T	T
A	T	A	T	A	T	A	A	T	T	A	T	T	C	T	T	T	G	C	A	T	A	T	T	A	T	T
A	T	A	T	T	C	T	A	T	C	A	A	C	A	C	C	C	G	T	A	T	A	T	T	A	C	T
A	A	A	T	C	T	T	T	T	T	A	C	T	A	T	A	T	G	T	G	A	G	T	T	A	T	A
A	T	A	T	A	C	A	A	T	T	A	T	T	C	T	C	T	G	C	G	A	A	C	T	A	T	T
A	A	A	T	T	T	T	T	C	T	T	T	T	A	T	T	T	A	T	A	T	A	A	T	A	C	T
A	T	A	C	T	T	C	T	T	T	T	C	C	A	T	T	T	G	T	A	T	A	T	T	A	T	T
A	A	A	T	T	T	C	A	T	T	A	T	T	A	T	A	T	G	T	A	T	A	T	T	A	T	T
A	A	A	T	A	C	A	A	T	T	A	T	T	C	T	C	G	C	G	A	A	C	T	A	C	T	T
A	T	A	T	T	T	T	T	T	T	T	C	T	A	T	T	T	G	T	A	C	A	T	T	A	T	C

Fig. 1 The combination of nucleotide diagnostic sites (identification characteristics, ICs) on COI gene fragments of 12 species of *Dendroctonus*

The length of COI gene fragment is 325 bp. The IC was generated by the software GBAS. Each of the 12 species can be represented by a unique IC.

4 DICUSSION

Although universal primers are frequently used in DNA barcoding (*e. g.*, LCO1490 and HCO2198; "Jerry" and "Pat") (Folmer *et al.*, 1994; Simon *et al.*, 1994), sometimes the PCR is not always successful. Therefore, we designed genus-specific primers that amplify a shorter DNA seqeunce to improve amplification efficiency in *Dendroctonus*, which has become a major threat to coniferous forests in China. To accurately identify the specimens intercepted at ports, we employed a suite of nucleotide diagnostic sites as the IC, which is generated by GBAS. Each species is represented by a unique IC. The ICs of unidentified samples were compared to the IC database stored in the Plant Quarantine Organisms Identification System. We demonstrated that our method can accurately identify samples of *Dendroctonus*.

Because the substitution rate of mitochondrial DNA could vary among species, genetic distance within one species can be greater than interspecific distance among other species. This violates the expectation by distance-based barcoding methods, which rely on a "barcoding gap" (Meyer and Paulay, 2005) to assign unknown samples to species. On the other hand, character-based method like IC here does not depend on a genetic distance threshold. Closely related species are clearly differentiated through the detection of species-specific nucleotide sites (Rach *et al.*, 2008; Yassin *et al.*, 2010; Goldstein and DeSalle, 2010).

For insect groups that consist of a large number of species, sampling priority should be given to species that share similar morphology or with different geographical

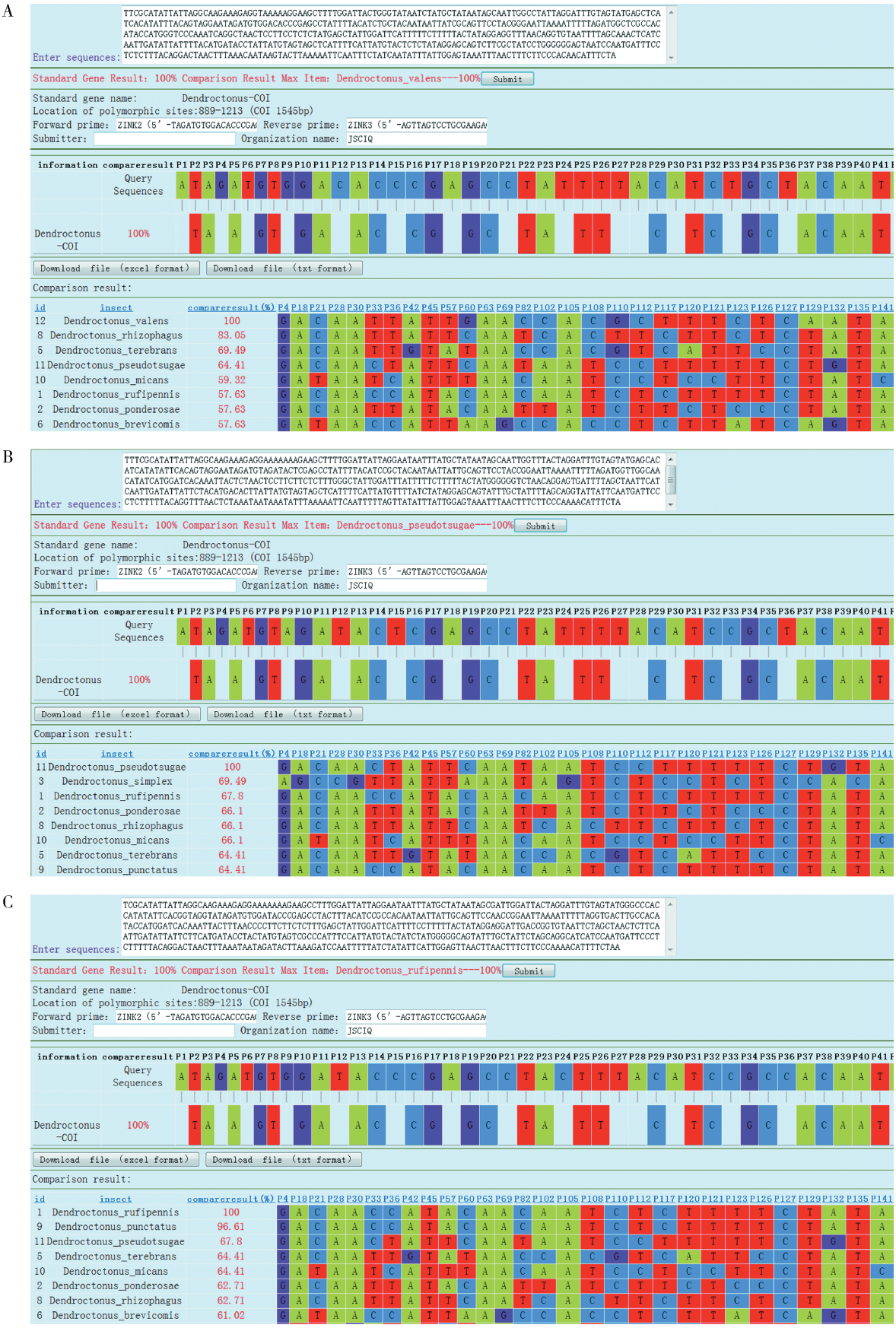

Fig. 2　Comparison of query sequence of unidentified *Dendroctonus* samples to the IC database

A: IC comparison for sample #1 and #2; B: IC comparison for sample #3; C: IC comparison for sample #4, #5 and #6. When samples belong to the same species, their IC comparison results will be identical.

races or host species. By narrowing down the detection range, we can improve the accuracy of species identification. Moreover, public database (*e. g.*, GenBank) should be fully utilized to ensure a high coverage of sample number in the targeted group, which will cover the most intraspecific and interspecific variation available. This will further increase the accuracy of DNA barcoding.

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DNA 条形码试剂盒检测技术在大小蠹属种类鉴定中的应用

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摘要: 【目的】DNA 条形码技术已成为生物分类鉴定的有力工具。DNA 条形码技术的相关问题,如物种种内和种间的遗传距离出现重叠区域,将直接影响到物种鉴定的准确性。我们应用 DNA 条形码试剂盒检测技术来快速、准确地鉴定口岸截获的检疫性大小蠹属种类。【方法】针对大小蠹昆虫设计引物以提高 PCR 扩增效率。运用自主研发的基因条码分析软件找出基因片段上区分每个物种的多态位点规律,作为该物种的鉴定特征并建立数据库,应用于物种鉴定。【结果】使用针对大小蠹属昆虫设计的引物成功扩增出 325 bp 的 COI 基因片段。将大小蠹属 12 种昆虫的 COI 基因片段上的核苷酸诊断位点的组合作为物种的鉴定特征,可以准确地区分近似种。通过比对植物检疫鉴定系统数据库里的鉴定特征,将 6 个大小蠹属的未知样品成功鉴定到种(核苷酸序列一致性为 100%),与形态鉴定结果一致。【结论】结果表明 DNA 条形码试剂盒检测技术可以准确鉴定大小蠹属的种类。该检测技术可以应用于其他经济重要性有害生物的检测鉴定。

关键词: 树皮小蠹; 入侵物种; DNA 条形码; COI; 鉴定特征; GBAS

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